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Original Article

Clinical Features and HLA Association of 5-Aminosalicylate (5-ASA)-induced Nephrotoxicity in Inflammatory Bowel Disease

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Abstract

Background and Aims: Nephrotoxicity is a rare idiosyncratic reaction to 5-aminosalicylate (5-ASA) therapies. The aims of this study were to describe the clinical features of this complication and identify clinically useful genetic markers so that these drugs can be avoided or so that monitoring can be intensified in high-risk patients.

Methods: Inflammatory bowel disease patients were recruited from 89 sites around the world. Inclusion criteria included normal renal function prior to commencing 5-ASA, $\geq 50\%$ rise in creatinine any time after starting 5-ASA, and physician opinion implicating 5-ASA strong enough to justify drug withdrawal. An adjudication panel identified definite and probable cases from structured case report forms. A genome-wide association study was then undertaken with these cases and 4109 disease controls.

Results: After adjudication, 151 cases of 5-ASA-induced nephrotoxicity were identified. Sixty-eight percent of cases were males, with nephrotoxicity occurring at a median age of 39.4 years (range 6–79 years). The median time for development of renal injury after commencing 5-ASA was 3.0 years (95% confidence interval [CI] 2.3–3.7). Only 30% of cases recovered completely after drug withdrawal, with 15 patients requiring permanent renal replacement therapy. A genome-wide association study identified a suggestive association in the HLA region ($p = 1 \times 10^{-7}$) with 5-ASA-induced nephrotoxicity. A sub-group analysis of patients who had a renal biopsy demonstrating interstitial nephritis ($n = 55$) significantly strengthened this association ($p = 4 \times 10^{-9}$, odds ratio 3.1).

Conclusions: This is the largest and most detailed study of 5-ASA-induced nephrotoxicity to date. It highlights the morbidity associated with this condition and identifies for the first time a significant genetic predisposition to drug-induced renal injury.

Key Words: 5-Aminosalicylates; nephrotoxicity; renal failure pharmacogenetics; stratified medicine; ulcerative colitis

1. Introduction

5-Aminosalicylate (5-ASA) medications are the most frequently prescribed class of drug to induce and maintain remission in patients with mild to moderately active ulcerative colitis.¹ Originally administered in combination with sulfapyridine as sulfasalazine, 5-ASA is now more often coated with a resin/gel or as a pro-drug/dimer to enhance distal bowel delivery through preparations such as mesalazine, olsalazine and balsalazide.² The use of these agents in maintenance therapy over decades means that long-term toxicity is an important consideration.

Nephrotoxicity associated with 5-ASA agents was first described in animal models and case reports in the 1970s and has since been reported multiple times for both sulfasalazine and the more modern 5-ASA agents.^{3–8} In 1990 the UK Committee on Safety of Medicines issued an alert on nephrotoxic reactions to mesalazine.⁹ Data from clinical trials suggest an annual risk of 0.26% and data from a questionnaire sent to gastroenterologists estimated an incidence of 1 case per 4000 patient years.^{10,11} A review of the UK General Practice Research Database calculated the incidence at 0.17 cases per 100 patients per year but the authors noted that only 13% of these patients had a histological diagnosis of interstitial nephritis.¹² Regular monitoring of renal function for the duration of therapy is recommended, although the cost-effectiveness of this approach has not been demonstrated.

Rare idiosyncratic drug reactions are often notoriously difficult to characterize due to the small number of cases available to individual researchers. The International Serious Adverse Events Consortium was launched in 2007 to facilitate the collection of large cohorts of patients who developed these rare serious drug side effects.¹³ Members of this consortium have recently demonstrated the utility of using small numbers of well-characterized cases to identify strong, clinically useful genetic risk factors for serious adverse drug reactions through genome-wide association study methodologies. Good examples of this approach are the identification of HLA-B*57:01 as a major determinant of cholestatic liver injury associated with flucloxacillin and our recent identification of an association between HLA-DRB1*07:01 and thiopurine-induced pancreatitis.^{14,15}

In this study we describe in detail, for the first time, a cohort of patients with inflammatory bowel disease (IBD) who developed nephrotoxicity subsequent to 5-ASA administration. We use this cohort to characterize the clinical features of this serious adverse event and then perform the first genome-wide association study to identify genetic risk factors for the development of drug-induced renal injury.

2. Methods

2.1. Patient recruitment

Individual study sites identified and recruited patients with 5-ASA-induced nephrotoxicity. This study was open to recruitment at 118 UK research sites (73.8% of the 160 acute NHS trusts in the UK) as well as 45 international sites. In total 77 sites from the UK and 12 sites from outside the UK recruited one or more patients. The protocol was approved by the National Research Ethics Committee South West, Exeter, UK (10/H0203/76) and by all local research and development offices.

Inclusion criteria for patient recruitment required the presence of all of the following:

- Patient administered any 5-ASA compound for treatment of inflammatory bowel disease (Crohn's disease, ulcerative colitis or IBD unclassified).

- Patient aged 6 or over.
- Normal creatinine or estimated glomerular filtration rate (eGFR) prior to first administration of 5-ASA or a creatinine that returned to the normal range after cessation of therapy.
- Rise in serum creatinine $\geq 50\%$ any time after introduction of 5-ASA.
- Physician opinion implicating 5-ASA strong enough to justify drug withdrawal, even if temporary.

Cases were identified from recruiting sites through clinics and systematic searches of historical records and pathology databases. Gastroenterologists who replied to a 2001–2002 UK survey of 5-ASA-induced nephrotoxicity were encouraged to submit cases.¹⁰ We invited clinicians who had submitted adverse drug reaction reports to the Medicines and Healthcare Products Regulatory Agency (MHRA) to consider recruiting patients. We also undertook direct advertising to patients through the national patient newsletter. Cases were recruited who developed nephrotoxicity between 1988 and 2013 (73% of cases were diagnosed with renal injury after the year 2000).

2.2. Case adjudication

An anonymized case report form detailing demographic, clinical and drug history was completed with the aid of hospital records. Two 6-mL EDTA blood samples were taken at this visit for DNA extraction (BD Vacutainer, USA). The case report forms also requested creatinine levels and their corresponding dates at four time points: (1) at baseline (usually before 5-ASA commenced, but not exclusively); (2) at the recording of the first abnormal creatinine value; (3) the worst creatinine value; and (4) the best recovered creatinine value. After data collection, the last normal creatinine value before development of renal injury was also obtained, if it was available (92/151 cases), to enable better characterization of the time period. If a renal biopsy was performed, the anonymized report was requested.

To assess patient eligibility for entry to this study, at least three gastroenterologists and at least one nephrologist reviewed each case for causality at a dedicated in-person adjudication panel meeting. For each case the evidence implicating 5-ASA as the cause of nephrotoxicity was assessed using an adapted version of the validated Liverpool Adverse Drug Reaction Causality Assessment Tool, displayed in [Supplementary Figure 1](#).¹⁶ Patients were classified as definite, probable, possible or unlikely cases of 5-ASA nephrotoxicity based on the adjudicator's independent assessment that the nephrotoxicity was due to 5-ASA treatment. The panel discussed all cases before a final adjudication decision was reached. Only individuals classified as probable or definite cases of 5-ASA-induced nephrotoxicity were taken forward for clinical and genetic analyses.

Concomitant administration of any medications known to cause nephrotoxicity classified the patient as a possible case and these cases were excluded. This included the use of (1) antibiotics (penicillins, cephalosporins, ciprofloxacin, sulphonamides, rifampicin); (2) diuretics (furosemide, bumetanide, thiazides); (3) non-steroidal anti-inflammatory drugs; (4) proton pump inhibitors; (5) allopurinol; (6) cimetidine; and (7) indinivir. The presence of uncontrolled diabetes, uncontrolled hypertension or peripheral vascular disease also classified the patient as a possible case of 5-ASA-induced nephrotoxicity and such patients were not taken forward for analysis. A patient treated with 5-ASA for microscopic colitis was recruited in error but was excluded during the adjudication process.

Definite cases required the development of renal injury upon rechallenge with 5-ASA. Cases classified as probable demonstrated

a temporal relationship with 5-ASA administration with no other identifiable risk factors for renal injury as described above.

2.3. DNA extraction and genotyping

DNA was extracted from EDTA-stabilized blood using the Qiagen Autopure LS with Puregene chemistry. Samples were genotyped on the Illumina Infinium HumanCoreExome beadchip (Illumina, USA), which contains 264 909 haplotype-tagging single-nucleotide polymorphism (SNP) markers, and 244 593 exome-focused markers by the Broad Institute (Boston, USA).

2.4. Clinical data analysis

Paper case-report forms were entered into the electronic database before independent, two-person, unblinded data cleaning was undertaken to ensure data quality prior to analysis. All data were analysed in R 3.0.2.¹⁷ Listwise deletion was used for missing data. Normality was tested for by the Shapiro–Wilks normality test ($p < 0.05$ considered non-parametric). Non-parametric data are presented with the median value and the 95% confidence interval (CI) of the median, with comparisons performed by a Wilcoxon signed rank test or χ^2 test as appropriate. Logistic and multivariate regression was conducted in R with pre-set variables (no stepwise regression). The definition of a return to baseline creatinine was a return to the upper limit of the local hospital laboratory's normal range or 120 $\mu\text{mol/L}$ if this range was not known.

2.5. Genome-wide and HLA imputation and association analyses

Three samples failed DNA extraction and were unavailable for genetic analysis. Genotyping was performed on the remaining 148 cases adjudicated as definite or probable 5-ASA-induced nephrotoxicity using the HumanCoreExome SNP Chip. Genotypes were called using Gencall.¹⁸ We excluded SNPs with an Hardy-Weinberg Equilibrium [HWE] $p < 0.0001$ and a genotype success rate < 0.99 . We excluded indels. Exclusion criteria for case samples were a genotyping success rate < 0.98 and a heterozygosity rate > 4 SDs (no samples were removed based on these criteria). To improve calling of low-frequency variants we used zCall.¹⁹ After running zCall, SNPs were excluded if they had a HWE $p < 0.0001$, Minor Allele Frequency [MAF] < 0.01 or if they were duplicated. This left 264 088 autosomal SNPs for imputation. The control patients with Crohn's disease and ulcerative colitis were obtained from the UK IBD Genetics Consortium as part of the Wellcome Trust Case Control Consortium (WTCCC 1 for Crohn's disease and WTCCC 2 for ulcerative colitis).^{20,21} There were 1748 Crohn's disease control samples genotyped on the Affymetrix 500K SNP chip and 2361 ulcerative colitis samples genotyped on the Affymetrix 6 SNP chip available for this analysis. Preliminary quality control (QC) had already been performed on the 1748 Crohn's disease and 2361 ulcerative colitis samples.^{20,21} From these two control cohorts, we excluded SNPs with a genotyping success rate < 0.99 , MAF < 0.01 and a HWE $p < 0.0001$. This left 396 255 (Crohn's disease) and 727 195 (ulcerative colitis) autosomal SNPs. To exclude ethnic outliers we performed principal components analysis using genome-wide complex trait analysis (GCTA).²² To generate the principal components we used a set of 36 702 SNPs that were imputed with $R^2 > 0.99$ in the cases (see below) and directly genotyped in the two control cohorts and were not in strong linkage ($r^2 < 0.2$).

Four 5-ASA nephrotoxicity cases and 62 control samples were excluded for being > 4 SDs from the first or second principal components. We used Kinship-based INference for Gwas [KING]²³ to test for cryptic relatedness between samples. If a case and control pair of samples had a kinship coefficient > 0.2 we excluded the control

sample, otherwise we excluded one of the pair of samples at random. One case sample and 13 control samples were excluded because of relatedness to other case or control samples. After exclusions this left 143 'probable' and 'definite' cases. The ratio of Crohn's disease to ulcerative colitis patients in the control group (59%) was similar to that in the case cohort (60%).

As previously,¹⁵ we used minimac²⁴ to impute into the European phase 1 version 3 (20101123) SNP and indel reference panel to prevent spurious associations due to variations in genotyping chips between cohorts. Seventy-six percent of the 9 412 474 SNPs with MAF $> 1\%$ frequency were imputed at $R^2 > 0.6$ in the cases, 75% in the Crohn's disease controls and 82% in the ulcerative colitis controls. As a different SNP genotyping chip was used for each of the three case and control cohorts, we focused subsequent association analyses on a very conservative subset of 2 883 071 SNPs that had an imputation $R^2 > 0.95$ in all three cohorts. For dedicated imputation of the HLA region we used SNP2HLA²⁵ and imputed into the T1DGC reference panel of 5224 individuals that have had classical HLA alleles typed as well as SNPs and indels by the immunoChip. Of the 8961 variants in the T1DGC panel, 8398 were captured with an INFO score > 0.8 . Mach2dat²⁶ was used to perform association analyses for the genome-wide analyses and PLINK²⁷ was used to perform association analyses for the HLA imputed analyses.

2.5. Data access

Phenotype and genotype data for cases are freely available upon request from the iSAEC Data Access Committee for users who comply with the Consortium's Data Release and IP Policy.¹³ Data will be available from <https://dataportal.saeconsortium.org/> within 12 months of genotype completion. Raw genotype data are freely available to researchers upon request. For further data access details please contact: saec@c2b2.columbia.edu.

Genotype data for the WTCCC ulcerative colitis and Crohn's disease cases are available from the European Genome-Phenome Archive at <https://www.ebi.ac.uk/ega/home>.

3. Results

3.1. Patient identification and adjudication

Through our international network of research sites we recruited a total of 204 IBD patients with suspected 5-ASA-induced nephrotoxicity. All cases underwent a rigorous assessment of causality by an adjudication panel composed of nephrologists and gastroenterologists using a validated tool.¹⁶ After this panel meeting the development of nephrotoxicity could be confidently assigned to the administration of 5-ASA medications for 151 out of 204 patients. Of these 151 cases, 5 were classified as definite cases of 5-ASA-induced nephrotoxicity, as they had a second episode of kidney injury when re-challenged with the agent. The remaining 146 cases were classified as probable cases.

3.2. Clinical features of 5-ASA-induced nephrotoxicity

The 151 patients who were adjudicated as definite or probable cases comprised 58 patients with Crohn's disease, 88 patients with ulcerative colitis and 5 patients with IBD unclassified. Sixty-eight percent of cases recruited were male. The median age at diagnosis of Crohn's disease was 29.5 years (95% CI 25.2–33.9) while the median age for ulcerative colitis was 29.7 years (26.7–32.8). One hundred and forty-six patients (97%) self-identified as being of white ethnicity, 2 patients did not provide a reply while 3 patients reported mixed

ancestry. A summary of the disease activity and location in the 2 years prior to development of renal disease is shown in Table 1.

The median duration of 5-ASA treatment prior to first detection of a raised creatinine was 3.0 years (95% CI 2.3–3.7). An abnormal creatinine was reported in 13% of cases within the first 12 months of treatment. For 27% of patients we were unable to find a creatinine between the initiation of the drug and the first abnormal creatinine measurement. In these patients the median time from starting the drug to detection of nephrotoxicity was 4 years (95% CI 2.73–5.28) while the cohort with interval measurements had a median time of 2.5 years (95% CI 1.70–3.36, $p = 6 \times 10^{-4}$). There was no significant difference in creatinine level at detection of abnormal renal function ($p = 0.75$) or in the rate of recovery ($p = 0.72$) between these two groups.

The majority of patients (91%) received oral 5-ASA alone with an average daily dose of 2.3 g (95% CI 2.1–2.5). Twelve patients received a mix of oral and rectal 5-ASA, while 1 patient received only rectal 5-ASA preparations. The majority of patients were treated with mesalazine (Supplementary Table 1); however, most available 5-ASA preparations are represented in the data. Figure 1 demonstrates the median creatinine levels and the time period at which these levels were collected at each time point for all 151 patients who developed nephrotoxicity. The density estimates for this plot are shown in Supplementary Figures 2 and 3.

Forty-five patients (30%) demonstrated full recovery of renal function within the follow-up period (median follow-up period 5.10 years, 95% CI 4.17–6.02). We undertook a multivariate regression analysis to investigate whether any clinical features were predictive of renal function recovery after 5-ASA cessation (Supplementary Table 2). This analysis suggested that the length of 5-ASA treatment ($p = 0.05$) and the average dose of 5-ASA ($p = 0.03$) inversely correlated with the likelihood of renal function recovery, suggesting cumulative toxicity. It has been suggested that patients who develop nephrotoxicity and stop the agent within 10 months of starting are more likely to recover to a normal creatinine.²⁸ We were unable to replicate this association in our data ($p = 0.53$); however, patients who did recover renal function appeared to have been taking 5-ASA for a shorter period of time before developing nephrotoxicity (median 794 days, 95% CI 459.13–1128.87 vs median 1461 days, 95% CI 1008.80–1597.20, $p = 0.02$; Figure 2). Forty-three percent of patients were treated with steroids, which was not associated with a shorter time to recovery ($p = 0.20$) or an increased rate of recovery ($p = 0.10$).

Fifteen out of 151 patients (9.9%) received renal replacement therapy, which for 13 patients took the form of a renal transplant. The remaining two patients were dialysis-dependent at the date of study end. A multivariate regression analysis identified peak

creatinine ($p = 0.008$), treatment with steroids ($p = 0.037$) and presence of a renal biopsy ($p = 0.045$) as predictive of the need for renal replacement therapy (Supplementary Table 3).

In total 76 patients adjudicated as definite or probable underwent a renal biopsy, 57 of which demonstrated clear evidence of interstitial nephritis. Of these, 58% demonstrated only chronic inflammatory changes in the interstitium, with 22 and 20% demonstrating acute or acute on chronic interstitial inflammation respectively. The remaining 19 biopsies demonstrated evidence of glomerulosclerosis or were non-diagnostic.

3.3. Genetic determinants of 5-ASA-induced nephrotoxicity

The idiosyncratic nature of 5-ASA-induced nephrotoxicity suggests that there may be a genetic basis for its development. To investigate this we undertook a genome-wide association study with the 151 cases described above and a control cohort of 1748 Crohn's disease and 2361 ulcerative colitis cases.

The strongest association signal for the development of nephrotoxicity was in the HLA region (rs3135349, odds ratio 2.04, $p = 1 \times 10^{-7}$) (see Figure 3 for Manhattan and QQ plots). We therefore performed dedicated HLA imputation using SNP2HLA in the T1DGC reference panel. The top SNP after imputation was rs3135356 (odds ratio 2.0, 95% CI 1.55–3.10, $p = 1 \times 10^{-7}$). The results are shown in Table 2. This association was present in patients with Crohn's disease and ulcerative colitis when tested independently ($p = 1 \times 10^{-7}$ and $p = 3 \times 10^{-7}$ respectively).

We recognize that despite the strict adjudication methods undertaken to assign causality of nephrotoxicity to the administration of 5-ASA compounds, there might be other factors that we have not captured in our data collection that could impact on renal function. To further refine the phenotype definition we therefore studied only those samples classified as definite or probable, who also had a renal biopsy demonstrating interstitial nephritis (passed genetic QC, $n = 55$). Limiting the association analyses to the biopsy-positive cases significantly strengthened the HLA association signal, despite the smaller number of cases, with the most associated SNP remaining rs3135356 (Figure 4), but with an odds ratio of 3.1, and a genome-wide significant p -value ($p = 4 \times 10^{-9}$). This was robust to correction for the first 20 principle components (odds ratio 3.13, $p = 1 \times 10^{-8}$). The most associated HLA allele from this analysis was HLA-DRB1*03:01 ($p = 5 \times 10^{-7}$, odds ratio 2.76). This variant was not associated with duration of therapy prior to the development of nephrotoxicity or the likelihood of recovery ($p = 0.63$ and $p = 0.22$ respectively).

Table 1. Montreal classification of disease location and severity in the 2 years prior to development of nephrotoxicity in 151 5-ASA nephrotoxicity cases.

Ulcerative colitis ($n = 93$)				Crohn's disease ($n = 58$)			
Extent		Severity		Location		Behaviour	
E1	5.5%	S0	14.0%	L1	5.2%	B1	81.1%
E2	20.9%	S1	36.6%	L2	56.9%	B2	17.0%
E3	69.2%	S2	45.2%	L3	37.9%	B3	1.9%
Ex	4.4%	S3	4.3%	L4	0.0%		

Ulcerative colitis (UC) classification:⁴² E1, ulcerative proctitis; E2, left-sided UC; E3, extensive UC; Ex, unknown. S0, clinical remission; S1, mild UC; S2, moderate UC requiring steroid or immunomodulator; S3, severe UC requiring admission or colectomy.

Crohn's disease classification:⁴² L1, ileal; L2, colonic; L3, ileocolonic; L4, isolated upper. B1, non-stricturing, non-penetrating; B2, stricturing; B3, penetrating.

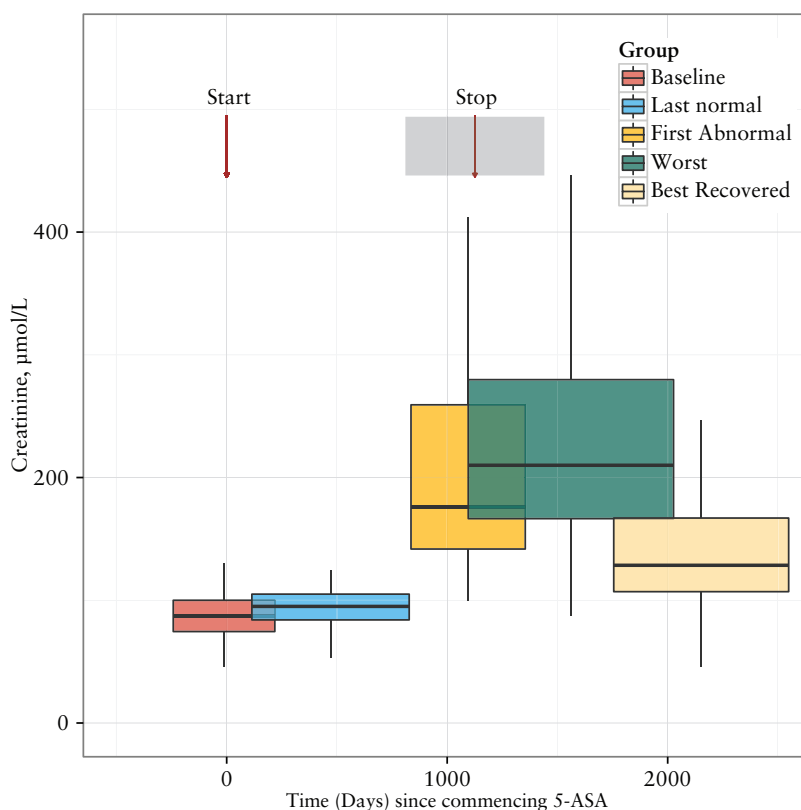


Figure 1. Boxplots for the cohort of 151 5-ASA-induced nephrotoxicity cases demonstrating time from treatment commencement with 5-ASA to development of nephrotoxicity and subsequent recovery. Serial boxplots demonstrating the median creatinine levels for $n = 151$ patients taken at each of the 5 study time points detailed in the Methods section (note $n = 92$ for last normal group). The position of the boxplot on the x-axis corresponds to the median time point from starting 5-ASA at which these creatinine values were obtained (the width of the boxplot is the 95% confidence interval of this value). The drug was started at time point 0 for all patients. The arrow labelled 'Stop' illustrates the median time at which the agent was stopped after commencement (the 95% confidence interval of this value is indicated by the grey box). Outlier values have not been visualized. Patients who underwent renal replacement therapy were excluded from the 'best recovered' group.

4. Discussion

We present here an analysis of the clinical features of patients with IBD who developed nephrotoxicity after administration of 5-ASA compounds. We have conducted the first ever genome-wide association study of drug-induced renal injury and gone on to identify a marker within the HLA region associated with 5-ASA-induced nephrotoxicity. The data from our cohort suggest that 5-ASA-induced nephrotoxicity may present at any age and is more common in male patients. The histological hallmark is a chronic tubulointerstitial nephritis. Renal injury was detected after a median treatment time of 3 years, following which only 30% of our cohort fully recovered renal function. In 10% of our cases, 5-ASA-induced nephrotoxicity necessitated permanent renal replacement therapy. These figures must be interpreted carefully, however, as the retrospective case identification methods used here may have led to recall bias, with greater recruitment of more severe cases.

Many drugs have been implicated in the development of interstitial nephritis, but proving causality is difficult. Nephrotoxicity induced by 5-ASA has been reported previously in case reports, including a case with a positive re-challenge.²⁹ These reports, combined with the 151 cases described here (including the 5 definite cases) provide compelling evidence that 5-ASA is able to cause renal injury and should be suspected in any patient with deteriorating renal function on these agents.

The temporal association between the use of 5-ASA and the development of renal injury, the improvement on drug withdrawal (although this only occurs in 30% of patients) and the 5 patients who were re-challenged with 5-ASA with subsequent worsening of renal function provide evidence that the renal damage is likely to be drug-related. The relationship between an increased likelihood of recovery and drug dose and duration also suggests a pathogenic role for the 5-ASA agents in interstitial nephritis development. However, there has been a suggestion that the nephrotoxicity observed in IBD patients might be an extra-intestinal manifestation of disease rather than a result of drug toxicity.^{12,30} Four of the patients in this study had evidence of granulomatous interstitial nephritis with non-caseating granulomas seen on biopsy (1 patient with Crohn's disease, 2 patients with ulcerative colitis and 1 patient with IBD unclassified). This rare form of interstitial nephritis is most commonly seen in acute drug reactions, but there are isolated case reports of patients with IBD developing interstitial nephritis with or without granulomas, some of whom have not been exposed to 5-ASAs.³¹⁻³³

Current British Society of Gastroenterology guidelines (2011) recommend monitoring of renal function annually in patients taking 5-ASA agents and the European Crohn's and Colitis Organisation (2012) recommends monitoring in high risk patients while the American Gastroenterology Society (2010) recommends periodic monitoring, noting that evidence for a defined frequency is lacking.³⁴⁻³⁶ The utility of these approaches has not been demonstrated;

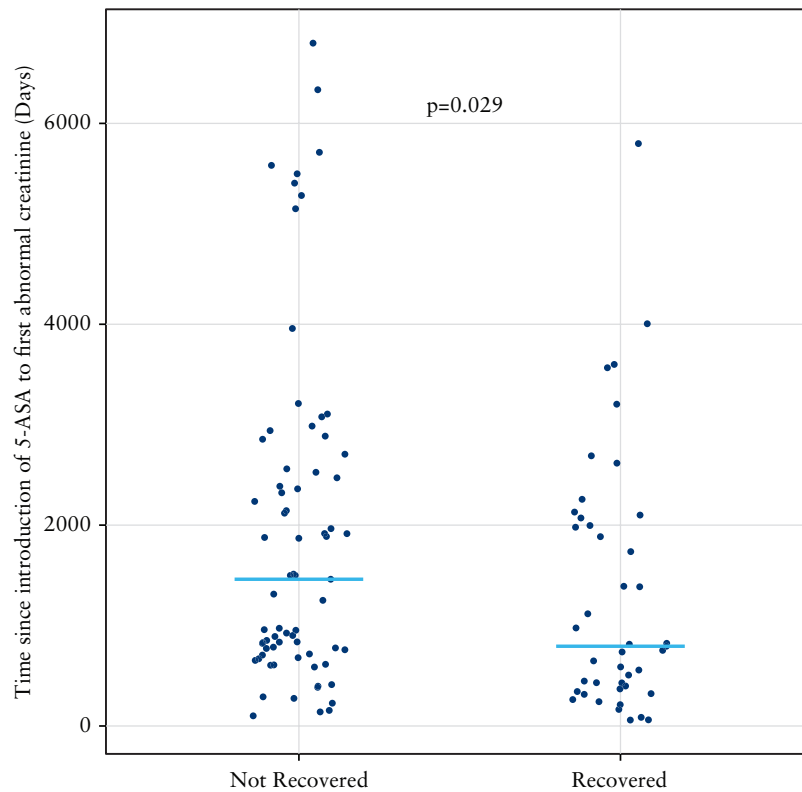


Figure 2. Time from initiation of 5-ASA agents to the development of nephrotoxicity and recovery of renal function to baseline level. This graph displays the time in days from initiation of 5-ASA agents to the development of nephrotoxicity by recovery status. The horizontal line illustrates the median for each cohort.

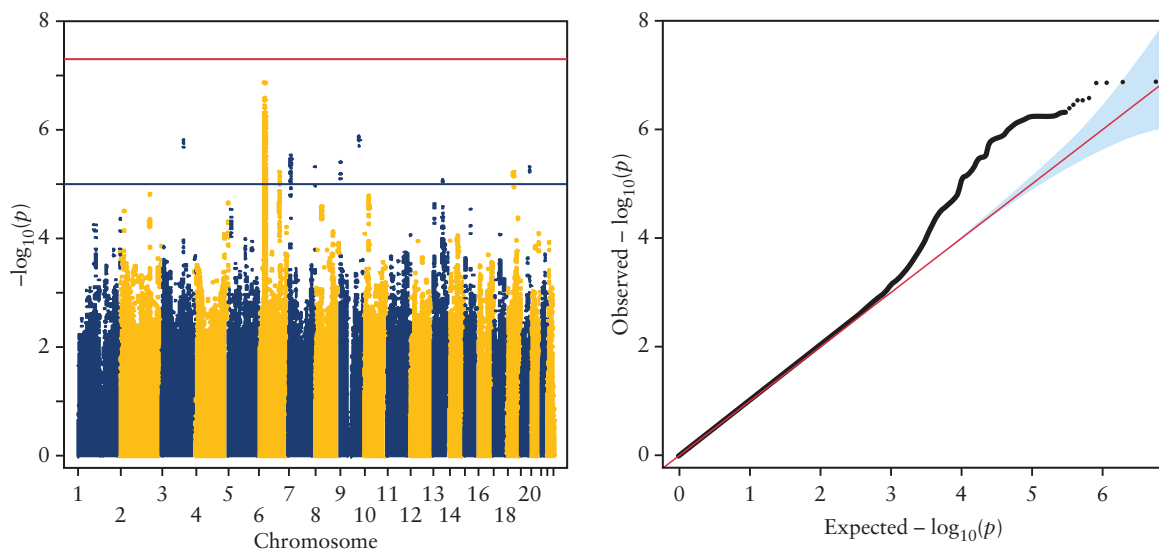


Figure 3. (A) Genome-wide Manhattan plot (including HLA imputation). Blue line, $p = 1 \times 10^{-5}$; red line, $p = 5 \times 10^{-8}$. (B) QQ plot (including HLA imputation).

however, it has been noted that many patients do not have regular renal function monitoring whilst using 5-ASA.³⁷ Indeed, data from this study suggest that the median time from the last normal creatinine to the first abnormal value, which represents how often a patient has a blood test, is 1.98 years, with a range of 2 days to 15.3 years.

5-ASA agents are normally tolerated by the majority of patients, suggesting an underlying genetic or environmental predisposition to the development of renal injury in a small subset of patients. The type of

renal injury seen with 5-ASA appears to be consistent with the changes occasionally seen with long-term lithium use.³⁸ Lithium ingestion over a prolonged period of time (usually >2 years) has rarely been associated with the development of a chronic focal interstitial cortical fibrosis with mononuclear cell infiltrate – a chronic interstitial nephritis.³⁹ Analogous to the renal injury seen with 5-ASA, this typically occurs after a prolonged period of drug exposure and, once identified by routine blood testing, often fails to improve even after drug withdrawal.⁴⁰

Table 2. Top genome-wide association study (GWAS) association signals from the combined GWAS and HLA imputation analysis.

Single-nucleotide polymorphism	Cohort	Chromosome	Position (hg19)	Effect allele	Control risk allele frequency	Risk allele frequency	Odds ratio (SE)	Odds ratio (95% confidence interval)	p-value
rs3135356	All	6	32391516	A	0.17	0.29	2.00 (0.13)		1 × 10 ⁻⁷
	Biopsy only					0.39		3.11 (0.19)	4 × 10 ⁻⁹
rs12204929	All	6	119396266	T	0.05	0.11	2.79 (0.20)		4 × 10 ⁻⁷
	Biopsy only					0.10		2.26 (0.34)	0.02
rs10488193	All	7	12274220	G	0.11	0.21	2.15 (0.15)		3 × 10 ⁻⁶
	Biopsy only					0.25		2.74 (0.23)	1 × 10 ⁻⁵

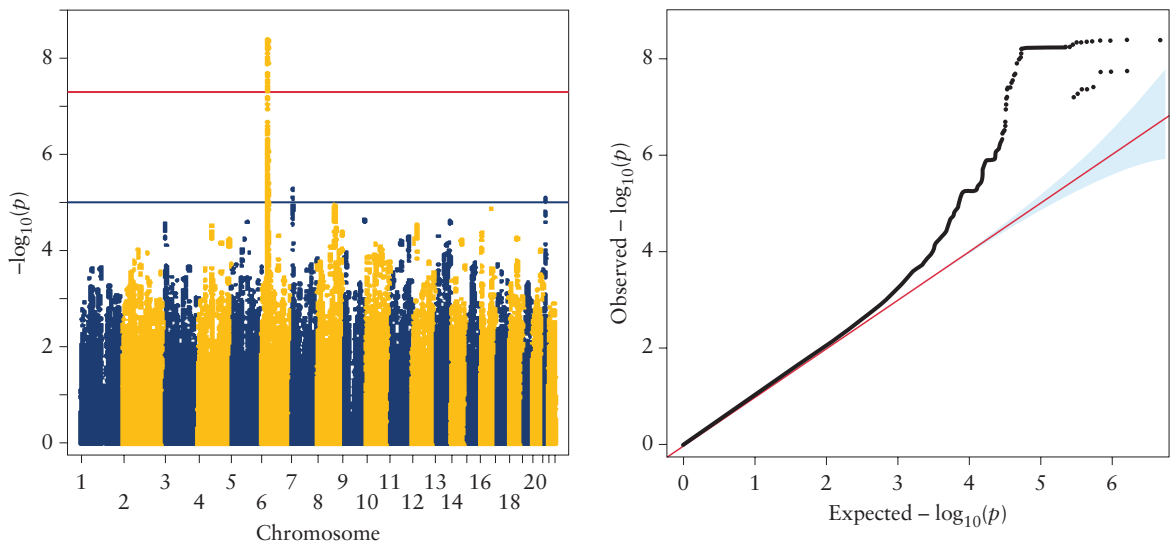


Figure 4. (A) Genome-wide Manhattan plot (including HLA imputation) excluding cases that did not have a renal biopsy demonstrating interstitial nephritis ($n = 55$) for all SNPs with MAF >0.05 . Blue line, $p = 1 \times 10^{-5}$; red line, $p = 5 \times 10^{-8}$. (B) Q-Q plot (including HLA imputation) excluding cases that did not have a renal biopsy demonstrating interstitial nephritis ($n = 55$).

We have not attempted to replicate the association of rs3135356 in an independent population. The collection of cases described here required a collection period of 2 years and the involvement of 89 centres, and consequently further sample collection was felt to be unfeasible. An association between another HLA class II allele, HLA-DRB1*01:02, and the rare syndrome of tubulointerstitial nephritis and uveitis (TINU) has been described in the literature.⁴¹ The association was only seen in patients with this syndrome and not in control patients with interstitial nephritis alone. This association is clearly distinct from the drug-induced renal injury displayed here and is likely to reflect their separate aetiologies.

Carriage of the risk allele is associated with a 3-fold increased risk of renal injury after 5-ASA administration. The high frequency of this SNP and the low frequency of the adverse event limits its clinical utility and we cannot recommend its use in guiding treatment choice or monitoring intervals.

We describe here an analysis of the clinical features of patients with IBD who developed chronic renal damage after administration of 5-ASA compounds. We have conducted the first ever association study of drug-induced renal injury and identify a genome-wide association with a class II allele. This rare condition appears to be more common in male patients, can occur after many years of drug administration and, once recognized, is only reversible in approximately one-third of patients. Although the cost-effectiveness of regular renal function measurements has yet to be demonstrated, the potential for serious long-term sequelae should necessitate its regular monitoring.

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Conflict of Interest

CWL has acted as a consultant to AbbVie, MSD, Takeda, Hospira, Pharmacosmos, Vifor Pharma and Dr Falk and received speaking fees and travel support from AbbVie, MSD, Takeda, Shire, Ferring, Hospira, Warner-Chilcott and Dr Falk. CJH has acted on advisory boards or as a consultant for Bayer Healthcare, InDex Pharmaceuticals, Novartis Consumer Health and Horizon Pharma. JM has done consultancy work for Genentech Inc., Tillotts Pharma, NAPP Pharmaceuticals Ltd and Takeda. TC has received honoraria and travel support from AbbVie, Ferring, Shire and Warner Chilcott. ML has received departmental financial support from Ferring and Warner Chilcott UK, speaker honoraria from MSD, travel support from Warner Chilcott UK and Vifor Pharma, and was a member of an advisory board for Vifor Pharma. IL has received educational grants and speaking endorsements and is a member of the advisory board for Shire, Freeing and Colazide. RD'I has received fees for

consultancy from AbbVie, MSD and Hospira. RP has received honoraria and consultancy fees from and is an advisory board member of Warner Chilcott, Dr Falk and Shire, and has received travel assistance from Warner Chilcott, Ferring and Dr Falk. PI has received honoraria for acting in an advisory capacity or speaking on behalf of AbbVie, MSD, Takeda, Genentech, Warner Chilcott, Ferring, Shire, Tillot's, Vifor and Pharmacosmos. DW has received honoraria, travel and educational grants from Shire, AbbVie, Warner Chilcott and Dr Falk. JH has received honoraria for lectures and consultancy from AbbVie, Ferring, Hospira, Medivir, MSD, Renapharma Vifor, Swedish Orphan and Takeda. FC has received lecture fees and travel support from Ferring Pharmaceuticals, Falk and Warner Chilcott. TKD has received lecture fees from Almirall. S. Sebastian has received research funding from AbbVie and MSD, honoraria for advisory board membership and speaker fees from Tillots Pharma, Ferring, Falk Pharma and AbbVie. MS has received research grants from Janssen, AbbVie and Prometheus and consulting and speaker fees from Janssen, AbbVie, Takeda and Prometheus. RG has received research support from AbbVie, Pharmatel Fresenius Kabi and Ferring, travel support from MSD, AbbVie, Falk and Schering-Plough and speaker fees from AbbVie, Janssen and Schering-Plough. SW has received speaker fees, sat on advisory boards for MSD and Abbott and received travel support from Ferring, MSD and Abbott. TO has received speaker fees or travel expenses or is a member of advisory boards for AbbVie, Dr Falk Pharma, Ferring UK, Merck, Napp, Warner Chilcott and Vifor Pharma. JB has received travel assistance from AbbVie. SCC has received travel assistance from AbbVie. RP has received travel assistance from Norgine. MP has received travel support and honoraria for lectures from Dr Falk Pharma and Warner Chilcott UK. DRG has received travel support and speaker fees from Ferring, Warner Chilcott UK, Shire, Tillots and Dr Falk Pharma. EW has received travel support from Dr Falk Pharma. GAH has received travel support from Dr Falk Pharma and Tillots Pharma UK and advisory board payments from AbbVie. TA has received unrestricted educational grants from Merck, AbbVie, Ferring, Warner Chilcott and speaker and advisory board honoraria from Merck, AbbVie, NAPP, Ferring, Warner Chilcott, Falk, Jansen and Takeda. ATC has received unrestricted educational support from MSD and Ferring, education/travel support from AbbVie, MSD and Dr Falk to attend international meetings and advisory board payments from MSD and Ferring. VA has received unrestricted research grants from Giuliani, Sofar and Ferring, speaker fees from Ferring, Nycomed and Chiesi and travel support from Giuliani, Ferring, Sofar and Chiesi. A. Hart has served as consultant, advisory board member or speaker for AbbVie, Atlantic, Bristol Meyers Squibb, Celltrion, Falk, Ferring, MSD, Napp Pharmaceuticals, Pharmacosmos, Shire and Takeda. AD has received honoraria and unconditional financial support for educational activities from Warner Chilcott (UK), AbbVie, Falk Pharmaceuticals UK, Shire Pharmaceuticals and Takeda. SL has received educational support/honoraria from AbbVie, MSD, Warner Chilcott, Shire, Ferring and Dr Falk Pharma. C. Murray has acted on advisory boards and received speaker fees from AbbVie and MSD. NKdB has participated in an advisory board for Merck, received speaker fees from AbbVie and has received unrestricted research grants from Dr Falk Pharma, FM. A. Holden, AS, CB, CJM, C. Mowat, DB, EVT, FH, GCS, GRS, KS, LS, MD, M. Thomas, M. Tremelling, MW, ND, NE, PD, RC, RD'S, RKW, RO, SG, S. Lewis, SM, S. Sen, TI and TS have no conflicts of interest to declare.

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Author Contributions

TA and A. Holden conceived the study. GAH, KS, NE, AS and TA collated submitted cases. CB project managed the study. GAH, KS, NE, AS, TA, TKD, A. Hart, IL, S. Lewis, CJM, RD'S, RO, TS and EW performed adjudication of submitted cases. GAH performed clinical data analysis. GAH and MW performed genetic analysis. VA, JB, DB, RC, ATC, SCC, TC, FC, NKHD, RD'I, TKD, MD, AD, ND, PD, DRG, RG, SG, JH, A. Hart, CJH, FH, TI, PI, S. Lal, IL, CWL, S. Lal, ML, SM, JM, C. Mowat, FM, C. Murray, TO, MP, R. Phillips, R. Pollock, GRS, S. Sebastian, S. Sen, A. Sharma, MS, LS, GCS, M. Thomas, M. Tremelling, ET, DW, SW and RKW submitted a substantial number of cases and aided drafting of the manuscript. GAH and TA wrote the manuscript, which was reviewed by all authors.

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